

Dean L. Engelhardt, *et al.*

Serial No.: 08/486,069

Filed: June 7, 1995

Page 2 (Third Supplemental Amendment Following Applicants' February 22, 1999  
Second Supplemental Amendment, their July 24, 1998 Supplemental Response and  
Their July 6, 1998 Amendment Under 37 C.F.R. §1.116 - March 29, 1999)

**AMEND THE ABOVE-IDENTIFIED APPLICATION AS FOLLOWS:**

**In The Title:**

Change the title of the invention to:

-- Processes for Nucleic Acid Hybridization Detection, Nucleic Acid  
Sequencing and Chromosomal Characterization -- .

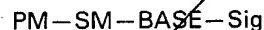
**In The Claims:**

Add new claims 376-400 as follows:

-- 376. (NEW) A process for determining whether the number of copies of a  
particular chromosome in a cell is normal or abnormal, the process comprising the  
steps of:

contacting said cell under hybridizing conditions with an oligo- or  
polynucleotide capable of hybridizing specifically to said particular chromosome,  
said oligo- or polynucleotide comprising at least one modified nucleotide selected  
from the group consisting of:

(i) a nucleotide having the formula



wherein

PM is a phosphate moiety,

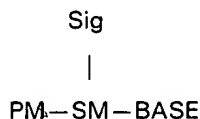
SM is a furanose moiety,

BASE is a pyrimidine, purine 7-deazapurine, and

Sig is a detectable moiety,

wherein PM is attached at the 3' or the 5' position of SM when said  
nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said  
nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM  
from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when  
BASE is a purine or a 7-deazapurine, and Sig is covalently attached to BASE  
at a position other than the C<sup>5</sup> position when BASE is a pyrimidine, at a  
position other than the C<sup>8</sup> position when BASE is a purine, and at a position,  
other than the C<sup>7</sup> position when BASE is a 7-deazapurine;

(ii) a nucleotide having the formula



wherein

PM is a phosphate moiety,

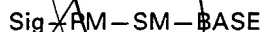
SM is a furanose moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety,

wherein PM is attached to SM at a position independently selected from the 2', 3', and 5' positions of SM when said nucleotide is a ribonucleotide, and at a position independently selected from the 3' and 5' positions when said nucleotide is a deoxyribonucleotide, BASE is attached to the 1' position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when BASE is a purine or 7-deazapurine, and Sig is covalently attached SM directly or through a linkage group; and

(iii) a nucleotide having the formula



wherein

PM is a phosphate moiety,

SM is a furanose moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is detectable moiety,

wherein PM is attached to the 3' or the 5' position of SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when BASE is purine, and Sig is covalently attached to PM,

to permit hybridization of said oligo- or polynucleotide to said particular chromosome;

detecting the signal generated by said hybridized oligo- or polynucleotide, thereby determining the number of copies of said particular chromosome; and

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comparing said determined number of copies of said particular chromosome  
with a number of copies of said particular chromosome determined for a normal cell  
containing said particular chromosome, thereby determining whether the number of  
copies of said particular chromosome in said cell is abnormal. --

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Cont

-- 377. (NEW) The process of claim 376, wherein said oligo- or polynucleotide  
comprises a clone derived from said particular chromosome. --

-- 378. (NEW) The process of claim 376, wherein said detecting step is carried  
out by a means selected from the group consisting of manual means and automatic  
means. --

-- 379. (NEW) The process of claim 378, wherein said manual means comprises  
visualization. --

-- 380. (NEW) The process of claim 378, wherein said automatic means  
comprises computerized automatic karyotyping. --

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-- 381. (NEW) A process for identifying a chromosome of interest in a cell  
containing other chromosomes, the process comprising the steps of:  
providing a set of clones specifically complementary to at least one sequence  
in said chromosome of interest, each of said clones comprising at least one  
modified nucleotide selected from the group consisting of:

(i) a nucleotide having the formula

PM-SM-BASE-Sig

wherein

PM is a phosphate moiety,

SM is a furanose moiety,

BASE is a pyrimidine, purine 7-deazapurine, and

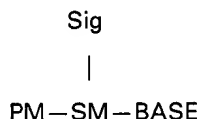
Sig is a detectable moiety,

Enz-5(D8)(C2)

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M8  
L1  
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wherein PM is attached at the 3' or the 5' position of SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when BASE is a purine or a 7-deazapurine, and Sig is covalently attached to BASE at a position other than the C<sup>5</sup> position when BASE is a pyrimidine, at a position other than the C<sup>8</sup> position when BASE is a purine, and at a position other than the C<sup>7</sup> position when BASE is a 7-deazapurine;

(ii) a nucleotide having the formula



wherein

PM is a phosphate moiety,

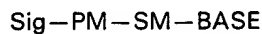
SM is a furanose moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety,

wherein PM is attached to SM at a position independently selected from the 2', 3', and 5' positions of SM when said nucleotide is a ribonucleotide, and at a position independently selected from the 3' and 5' positions when said nucleotide is a deoxyribonucleotide, BASE is attached to the 1' position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when BASE is a purine or 7-deazapurine, and Sig is covalently attached SM directly or through a linkage group; and

(iii) a nucleotide having the formula



wherein

PM is a phosphate moiety,

SM is a furanose moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is detectable moiety,

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wherein PM is attached to the 3' or the 5' position of SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when BASE is purine, and Sig is covalently attached to PM;  
fixing the chromosomes from or in said cell;  
contacting said fixed chromosomes under hybridizing conditions with said clones, permitting hybridization of said clones to said complementary sequence in said chromosome of interest;  
L1  
cont  
detecting any signal generated by each of said clones which have hybridized to its complementary sequence in said chromosome of interest, thereby obtaining a pattern of hybridizations between said set of clones and said chromosomes; and  
identifying said chromosome of interest by means of said hybridization pattern obtained. --

-- 382. (NEW) The process of claim 381, wherein said detecting step is carried out by a means selected from the group consisting of manual means and automatic means. --

-- 383. (NEW) The process of claim 382, wherein said manual means comprises visualization. --

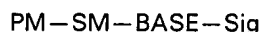
-- 384. (NEW) The process of claim 382, wherein said automatic means comprises computerized automatic karyotyping. --

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-- 385. (NEW) A process for identifying all of the chromosomes in a cell of interest, the process comprising the steps of:  
providing sets of clones, each of said set of clones being specifically complementary to at least one sequence in a chromosome of said cell of interest, each of said clones in said sets being labeled with a different indicator molecule and

each of said clones comprising at least one modified nucleotide selected from the group consisting of:

- (i) a nucleotide having the formula



wherein

PM is a phosphate moiety,

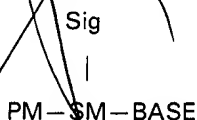
SM is a furanose moiety,

BASE is a pyrimidine, purine 7-deazapurine, and

Sig is a detectable moiety,

wherein PM is attached at the 3' or the 5' position of SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when BASE is a purine or a 7-deazapurine, and Sig is covalently attached to BASE at a position other than the C<sup>5</sup> position when BASE is a pyrimidine, at a position other than the C<sup>8</sup> position when BASE is a purine, and at a position other than the C<sup>7</sup> position when BASE is a 7-deazapurine;

- (ii) a nucleotide having the formula



wherein

PM is a phosphate moiety,

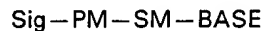
SM is a furanose moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is a detectable moiety,

wherein PM is attached to SM at a position independently selected from the 2', 3', and 5' positions of SM when said nucleotide is a ribonucleotide, and at a position independently selected from the 3' and 5' positions when said nucleotide is a deoxyribonucleotide, BASE is attached to the 1' position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when BASE is a purine or 7-deazapurine, and Sig is covalently attached SM directly or through a linkage group; and

(iii) a nucleotide having the formula



wherein

PM is a phosphate moiety,

SM is a furanose moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is detectable moiety,

wherein PM is attached to the 3' or the 5' position of SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when BASE is purine, and Sig is covalently attached to PM;

fixing the chromosomes from or in said cell;

contacting said fixed chromosomes under hybridizing conditions with said sets of clones, thereby permitting hybridization of said sets of clones to any of their complementary sequences in said chromosomes; and

detecting any signal generated by each of said different indicator molecules in said sets of clones which have hybridized to their complementary sequences in said chromosomes, thereby identifying each of said chromosomes in said cell of interest. --

-- 386. (NEW) The process of claim 385, wherein each of said set of clones is labeled with the same indicator molecule. --

-- 387. (NEW) The process of claim 385, wherein said detecting step is carried out by a means selected from the group consisting of manual means and automatic means. --

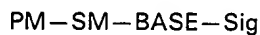
-- 388. (NEW) The process of claim 387, wherein said manual means comprises visualization. --

-- 389. (NEW) The process of claim 387, wherein said automatic means comprises computerized automatic karyotyping. --

-- 390. (NEW) A process for determining the number of chromosomes in an interphase cell of interest, the process comprising the steps of:

providing sets of clones, each of said set of clones being specifically complementary to at least one sequence in a chromosome of said interphase cell of interest and each of said clones in said sets comprising at least one modified nucleotide selected from the group consisting of:

- (i) a nucleotide having the formula



wherein

PM is a phosphate moiety,

SM is a furanose moiety,

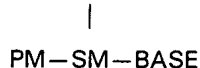
BASE is a pyrimidine, purine 7-deazapurine, and

Sig is a detectable moiety,

wherein PM is attached at the 3' or the 5' position of SM when said nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said nucleotide is a ribonucleotide. BASE is attached to the 1' position of SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when BASE is a purine or a 7-deazapurine, and Sig is covalently attached to BASE at a position other than the C<sup>5</sup> position when BASE is a pyrimidine, at a position other than the C<sup>8</sup> position when BASE is a purine, and at a position other than the C<sup>7</sup> position when BASE is a 7-deazapurine;

- (ii) a nucleotide having the formula

Sig



wherein

PM is a phosphate moiety,

SM is a furanose moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

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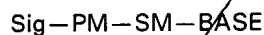
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Sig is a detectable moiety,

wherein PM is attached to SM at a position independently selected from the  
2', 3', and 5' positions of SM when said nucleotide is a ribonucleotide, and  
at a position independently selected from the 3' and 5' positions when said  
nucleotide is a deoxyribonucleotide, BASE is attached to the 1' position of  
SM from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when  
BASE is a purine or 7-deazapurine, and Sig is covalently attached SM directly  
or through a linkage group; and

(iii) a nucleotide having the formula



wherein

PM is a phosphate moiety,

SM is a furanose moiety,

BASE is a pyrimidine, purine or 7-deazapurine, and

Sig is detectable moiety,

wherein PM is attached to the 3' or the 5' position of SM when said  
nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when said  
nucleotide is a ribonucleotide, BASE is attached to the 1' position of SM  
from the N<sup>1</sup> position when BASE is a pyrimidine or the N<sup>9</sup> position when  
BASE is purine, and Sig is covalently attached to PM;

contacting said interphase cell under hybridizing conditions with said sets of  
clones, thereby permitting hybridization of said sets of clones to any of their  
complementary sequences in said chromosomes;

detecting any signals generated by each of said sets of clones hybridized to  
their complementary sequences in said chromosomes, to obtain a pattern of  
generated signals; and

comparing each generated signal with other generated signals in said pattern,  
thereby determining the number of chromosomes in said interphase cell of  
interest. --

-- 391. (NEW) The process of claim 390, wherein each of said sets of clones is  
labeled with the same indicator molecule. --

Enz-5(D8)(C2)

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-- 392. (NEW) The process of claim 390, wherein each of said sets of clones is  
labeled with a different indicator molecule. --

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-- 393. (NEW) The process of claim 390, wherein said detecting and determining  
step is carried out by a means selected from the group consisting of manual means  
and automatic means. --

-- 394. (NEW) The process of claim 393, wherein said manual means comprises  
visualization. --

-- 395. (NEW) The process of claim 393, wherein said automatic means  
comprises computerized automatic karyotyping. --

-- 396. (NEW) A process for detecting a nucleic acid of interest in a sample,  
which process comprises the steps of:

(a) hybridizing said nucleic acid of interest in the sample with an oligo-  
or polynucleotide comprising at least one detectable protein binding sequence  
capable of binding to said nucleic acid of interest; and

(b) detecting the presence of said detectable protein binding sequence,  
thereby detecting said nucleic acid of interest. --

-- 397. (NEW) The process of claim 396, wherein said at least one protein  
binding sequence is covalently attached to said oligo- or polynucleotide. --

-- 398. (NEW) The process of claim 397, wherein said covalent attachment  
comprises ligation. --

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conceded*  
-- 399. (NEW) The process of claim 396, wherein said at least one protein  
binding sequence is selected from the group consisting of a promoter, a  
repressor and an inducer. --

-- 400. (NEW) The process of claim 399, wherein said repressor comprises  
a *lac* repressor. --

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